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Target Organ Toxicity: Nervous System

by C. L. Mitchell*

This conference was organized in response to the resurgence of interest in the behavioral and neurological effects resulting from low-level exposures to a wide variety of chemical and physical agents. One factor in this renewed interest was the passage of the Toxic Substance Control Act. This law specifically states, among other things, that chemicals should be evaluated for their behavioral effects.

Unfortunately, real or potential risks to the nervous system are difficult to assess because of the complexity of the system. Some of the problems in assessment are associated with the wide variation in function which can occur and still lie within the classification of "normal." Some are associated with the plasticity of the nervous system. Other problems in assessment are related to our incomplete understanding of precisely what is being measured by certain tests. Clearly, no single test will suffice to examine the functional capacity of the nervous system. During the course of this conference, it became equally clear that the current state of our knowledge does not even warrant the selection of a battery of tests from the myriad procedures available. Dr. Dews stated, in his paper, that "no methods of predicting when prolonged exposure to a low level of an agent will lead to subtle and delayed behavioral effects in man have been validated." Elsewhere in this volume, Drs. Tilson and Cabe present one approach toward the validation of such methods.

Many behavioral toxicologists apparently either presume or hope that behavioral tests will prove to be more sensitive than other tests in detecting central nervous system toxicity. Dr. Norton reminded us, however, that "while the logical argument is easy to propose, critical experiments are rare in which careful behavioral studies have been paired with detailed morphological examination." To me, a more convincing argument for the use of behavioral tests, rather than sensitivity, is that given by Dr. Mello (*1*). She stated, "the behavior of the organism is the endpoint of the functional integration of the nervous system encompassing sensory, motor, and cognitive aspects. The functional capacity of the central nervous system cannot be determined by histological or even physiological studies independent of behavioral analysis." This theme was also espoused by Dr. Reiter in his introductory statements to this conference. Dr. Norton pointed out, however, that "it is the functioning of the central nervous system that is monitored by behavior, not damage for which compensation occurs or damage which affects only excess capacity or structural redundancy." It is thus clear that morphological, neurochemical, and neurophysiological techniques are likewise important if one is to fully examine the nervous system for toxic effects.

In the sessions on behavioral toxicology, Dr. Reiter pointed out the need for testing procedures which are not only sensitive indicators of toxicity but which are also simple, rapid, and inexpensive to perform. Dr. Laties discussed the ways in which operant conditioning can contribute to the development of behavioral toxicology. These were exemplified by the papers of Dews, Stebbins and Rudy, Evans, Annau, Wood, and Thompson and Moerschbaecher.

With respect to neurotoxicity, Dr. LeQuesne discussed the usefulness and limitations of electromyography in detecting the toxic effects of acrylamide, lead, organophosphates, hexacarbons, and methylmercury. Drs. Spencer and Schaumburg pointed out that there is no good systematic classification of neurotoxins because there is a paucity of information concerning the nature of their toxic effects. They also reminded us of the remarkable similarity between the pathological and clinical expression of certain distal axonopathies and naturally occurring neuropathies associated with diabetes, uremia, vitamin deficiencies, old age, and certain genetic-metabolic diseases. They pointed out that

* Laboratory of Behavioral and Neurological Toxicology, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709.

this mimicry suggests the possibility that exposure to neurotoxins might render a person more susceptible to or accelerate the onset of a naturally occurring peripheral neuropathy. Dr. Jacobs discussed the intriguing possibility that differences in vascular permeability in the nervous system might play a role in determining the selectivity of neurotoxins (methylmercury, for example) to particular parts of the nervous system. Dr. Krigman emphasized the importance of a quantitative approach to studying the morphological effects of toxins.

It was pointed out by Dr. Damstra that our knowledge of the primary biochemical events associated with neurotoxicity is very limited. She did, however, present an example of how knowledge of basic neurochemical events might contribute to the detection of neurotoxicity. This example had to do with the delayed neurotoxicity seen with some organophosphates. These organophosphates inhibit a nerve cell protein called neurotoxic esterase. She suggested it might be feasible to develop *in vitro* tests for determining whether a compound has this delayed neuropathy inducing property. Dr. Nelson discussed the feasibility of using neuronal cell cultures to assess an agent's effect on a variety of important neurobiologic parameters. Dr. Hanin gave a review of central neurotransmitter function and suggested that some environmental toxicants may exert their effects by altering central neurotransmitter function. Dr. Dunn presented a review of the neurochemistry of learning and memory and concluded with some suggestions as to neurochemical parameters to use in screening for neurochemical effects of toxicants.

The subject of variability and its consequencies on sample size and the ability to detect a statistically significant effect of a compound was discussed by Dr. Dews. This is a subject which, in my opinion, is too often ignored and stems in part, from a lack of understanding of the Type II error concept of statistics. I have dwelled on this at length elsewhere (2). A Type II error is made when the null hypothesis is accepted and it is, in fact, false. The probability of making such an error is called β. We seldom, if ever, know the value of β. We can say something about its relative magnitude, however. Its value depends upon: the distance between the population parameters being estimated by the samples (population means in the case of Student's t); the value selected for α (the probability of making a Type I error or rejecting the null hypothesis when it is, in fact, true); and the sample size. The smaller the distance between the population parameters, the larger will be β. β varies inversely with α. β decreases as sample size increases. Thus, if you want to detect a rather small effect and your experimental material is highly variable, you need a large sample size. In selecting the sample size, then, one must take these factors into consideration. You cannot escape answering the question of "how big a difference do I want to detect" (or "at what incidence of occurrence does that effect become a factor about which something should be done")? You either answer if directly or you answer it indirectly when you select your sample size. This is so because the smaller the sample size, the larger the change has to be in order to be statistically significant. There are techniques available which can tell you the sample size needed to detect either a given incidence of occurrence or a given change in magnitude if you have an estimate of the variability in the population(s). I cannot urge too strongly their use. Too many studies have been conducted with sample sizes so small that there has been no chance to detect any real but subtle effect.

Finally, I would urge that greater consideration be given to the reason(s) for conducting experiments in behavioral and neurological toxicity. In any emerging field, there is a certain amount of information which must be collected before concise hypotheses can be formulated. This does not, however, excuse the investigation from critically asking the questions of "what," "why," and "how." The lack of a solid data base is no excuse for ill conceived experiments. Indeed, the absence of information especially calls for critical thinking. Ill conceived experiments only create confusion and increase the amount of information which must be collected before intelligent decisions can be made. Science advances most rapidly when concise, meaningful hypotheses are tested. We need to be on guard, then, not simply to collect information but to continually sharpen our reasons for what we are doing.

REFERENCES

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2. Mitchell, C. L. The design and analysis of experiments for the assessment of drug interactions. Ann. N. Y. Acad. Sci. 281: 118 (1976).